cell numbers are reduced in the lungs when the gut and liver are inflamed.

If gut-liver axis alterations due to metabolic diseases are a key contributor to progression to severe COVID-19 then this information can be used to guide treatment of a large sector of society. Global prevalence rates are estimated at 24% for MAFLD, 13% for obesity and 8.5% for T2D. These subgroups are major contributors to the overall number of patients with COVID-19 that require hospitalisation (up to 25–35% in Western countries[10]). Trials of treatments that restore gut barrier integrity are also supported by this disease mechanism. For instance, therapeutics that have been developed for IBD, including probiotics and modulators of gut mucosal protection/regeneration, could reduce the number of patients with MAFLD/obesity/T2D that progress to severe COVID-19. Moreover, caution may have to be taken with drugs that disturb intestinal microbiota composition or abundance.

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Authors' contributions
NAY and GA wrote the letter, RW revised and approved the final version of the letter.

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Author names in bold designate shared co-first authorship

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Relevance of platelet-derived microvesicles in cirrhosis: The debate remains open

To the Editor:
We read with great interest the review by Thietart and Rautou on extracellular microvesicles in liver diseases.[1] They accurately describe the remaining challenges regarding both technical aspects and the relevance of microvesicle assessment for diagnostic and prognostic purposes. However, a couple of points deserve specific comment.

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Regarding the technical aspects: According to the authors, filtration should be preferred to centrifugation as a method to measure microvesicles. Indeed, they used filtration in their own studies.[2,3] However, the society of microvesicles does not recommend any one technique over another[4] and this point thus remains a matter of local practice. More importantly, whatever the method used, it is crucial to verify that the labelling used to identify the cellular origin of microvesicles is actually located in the membrane. To address this question,

exposure to a membrane detergent (*e.g.* Triton) appears to be mandatory. This is clearly stated in the recommendations from the society of microvesicles. This point appears particularly relevant in the field of liver diseases, where previous publications indicated that hepatocyte-derived microvesicles were increased in patients with more severe cirrhosis.[2,3] By reading these publications in depth, we noticed that the authors used cytokeratin-18 (CK18) labelling to identify hepatocyte-derived microvesicles but did not prove the membrane localization of CK18. Therefore, we cannot rule out that they detected only fragmented cellular debris from hepatocytes due to necrosis or apoptosis, thus reflecting "active cirrhosis", rather than authentic hepatocyte-derived microvesicles. Hence, their conclusion could only state that "active cirrhosis" confers poor prognosis, which makes perfect sense. In our experience, despite numerous attempts, we have failed to identify hepatocyte-derived microvesicles after CK18 labelling and Triton exposure in patients with cirrhosis. The CK18 signal constantly persisted, and to our knowledge, no published study has been able to assess hepatocyte-derived microvesicles using a careful method involving membrane detergent.

Regarding the clinical aspects: Because data regarding hepatocyte-derived microvesicles are difficult to interpret for the reasons mentioned, we believe that microvesicles of other origins deserve further investigation in patients with liver disease. Platelet-derived microvesicles are easy to detect, but their role in liver pathogenesis and/or outcomes remain unclear, due to conflicting results. In their review, the authors question the relevance of platelet-derived microvesicles in the setting of cirrhosis; they underline that the assessment and quantification of platelet-derived microvesicles depend on the markers used and are often hampered by technical issues during plasma sample preparation. Indeed, there are a variety of platelet-derived microvesicles. We conducted a pilot study comparing platelet-derived microvesicle levels in 90 patients with cirrhosis and 10 healthy volunteers using double immuno-labelling (*i.e.* CD41+/CD31− and phosphatidylserine membrane markers) and size discrimination ("small" platelet-derived microvesicles being identified by the use of beads <0.3 to 0.5 μm in diameter) of isolated microvesicles, to distinguish large and small platelet-derived microvesicles, and those expressing phosphatidylserine, which confers strong procoagulant properties.[5] As expected, the circulating levels of all subtypes of platelet-derived microvesicles were correlated with platelet counts. However, this correlation did not explain our findings regarding prognosis. Indeed, in our series, only 1 subtype of platelet-derived microvesicles, namely phosphatidylserine-positive, small platelet-derived microvesicles, had a prognostic impact: low levels of this platelet-derived microvesicle subtype were associated with lower 6-month transplant-free survival, independently of conventional prognostic scores.[6] This finding was still significant after sensitivity analyses removing patients with acute alcoholic hepatitis, systemic inflammatory response syndrome, acute kidney injury and/or acute-on-chronic liver failure. Conversely, low platelet counts and low levels of other platelet-derived microvesicle subtypes did not impact survival. Because plasma sample preparation was identical for the whole sample collection, it is unlikely that our results were biased by technical issues.

Our results suggest that small platelet-derived microvesicles expressing phosphatidylserine may contribute to liver damage. Since phosphatidylserine increases the procoagulant properties of platelet-derived microvesicles, one may hypothesize that low plasma levels of small platelet-derived microvesicles expressing phosphatidylserine are a consequence of their integration into microthrombi that could contribute to liver parenchymal extinction,[7] or intestinal damage favoring bacterial translocation,[8] with both phenomena affecting prognosis. From this perspective, our results, if confirmed, will reinforce the rationale for anticoagulant therapies patients with cirrhosis.[9] We therefore advocate for continued debate on platelet-derived microvesicles in cirrhosis.

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Authors' contributions

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Supplementary data

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